REMARKS

In response to the Final Office Action mailed February 24, 2004, Applicants have amended the claims, which when considered with the following remarks and enclosed exhibits, is deemed to place the present application in condition for allowance. Favorable consideration of all pending claims is respectfully requested.

In the first instance, Applicants through the undersigned, thank Examiner Collins for her time and consideration in granting a telephone interview on April 13, 2004. An Interview Summary was mailed to Applicants' representative on April 14, 2004. As set forth in the Interview Summary Record, the teachings of the prior art with respect to what was known regarding conservation of phosphorylation sites in plant CDKAs was discussed. In particular, the teachings of Joubes, M., et al., (2000) *Plant Molecular Biology 43*:607-620 submitted as Exhibit D with Applicants' response filed December 2, 2003, were discussed. Also discussed during the interview was the article by Schuppler et al. (previously provided as Exhibit C with Applicants' response filed December 2, 2003), in relation to the effects that water stress has on tyrosine phosphorylation in a Cdc2-like protein in wheat plants. The planned submission of references directed to what was known in the art about substituting non-phosphorylated amino acids other than phenylalanine and alanine for tyrosine and threonine respectively, was also discussed.

In the Office Action of February 24, 2004, claims 1, 5-6, 10-11, 18-20, 22-26, 28-29 and 31 remain rejected under 35 U.S.C. §112, first paragraph, as allegedly violative of the written description requirement. It is the Examiner's position that the presently claimed invention is allegedly not described in the application as filed because Applicants have only "disclosed" one nucleic acid molecule whose introduction into a plant confers

stress tolerance, that being a nucleic acid molecule encoding an *Arabidopsis* CDC2a (CDKA;1) mutein wherein the tyrosine at position 15 is substituted to phenylalanine and the threonine at position 14 is substituted to alanine. In response to this position of the Examiner, Applicants respectfully submit that the specification *discloses* different nucleic acid molecules encoding a CDK which may be introduced into a plant in order to confer stress tolerance. Applicants respectfully submit that Applicants have *exemplified* one nucleic acid molecule which when introduced into a plant confer stress tolerance, that being a nucleic acid molecule encoding an *Arabidopsis* CDC2a (CDKA;1) mutein wherein the tyrosine at position 15 is substituted to phenylalanine and the threonine at position 14 is substituted to alanine.

It is well-established that the law construing 35 U.S.C.§112, first paragraph, does not require a specific example of everything within the scope of the broad claims. *In re Anderson*, 471 F.2d 1237, 1240-41, 176 USPQ 331, 333 (CCPA 1973). In fact, the law does not require *any* specific working examples.

> If the Examiner and/or Board intended a rejection under the first paragraph of §112, it must be reversed inasmuch as the specification contains a statement of Appellant's invention which is as broad as Appellant's broadest claims...

In re Robbins, 429 F.2d 452, 456, 166 USPQ 552, 555 (CCPA 1970).

The Examiner also appears to predicate the written description rejection on the fact that "the specification does not describe or characterize other non-phosphorylatable amino acid residues that may occupy the positions corresponding to residues 14 and 15 respectively in *Arabidopsis thaliana* CDKA;1 as claimed in claims 1 and 5." Applicants

respectfully submit that one skilled in the art would be aware of other non-phosphorylatable amino acid residues that could be substituted for the phosphorylatable tyrosine or threonine, since this information was available in the prior art as of the priority date of the present application.

Exhibit A provides page 84 from *Molecular Biology of the Cell*, 2d ed., 1989, Bruce Alberts, et al., Garland Publishing, Inc., New York, N.Y. Specifically, under the heading "Enzymes Can Be Switched On and Off by Covalent Modification", the authors write: "[c]ells have different devices for regulating when longer lasting changes in activity, occurring over minutes or hours, are required. These involve reversible covalent modification of enzymes, which is often, but not always, accomplished by the addition of a phosphate group to a specific serine, threonine, or tyrosine residue in the enzyme. The phosphate comes from ATP, and its transfer is catalyzed by a family of enzymes known as protein kinases."

The present application, is directed to such a protein kinase. Specifically, the present invention is directed *inter alia*, to methods of using mutant cyclin dependent kinases (CDKs) comprising a PSTAIR cyclin binding motif, vectors comprising nucleic acid molecules encoding such mutant CDKs, and transgenic plants and plant parts comprising such nucleic acid molecules. The CDK muteins are not susceptible to inhibitory phosphorylation under certain stress conditions due to the substitution of a non-phosphorylatable amino acid in place of either a tyrosine at a position that corresponds to the tyrosine located at position 15 in the amino acid sequence of *A thaliana* CDKA;1, or for both this tyrosine and a threonine located at a position that

corresponds to the threonine located at position 14 in the amino acid sequence of *A. thaliana* CDKA;1.

Applicants further submit that as of the priority date of the present application, it was widely known which types of substitutions were more favorable than others, for the phosporylatable tyrosine or threonine. For example, Exhibit B provides pages from M.J. Betts, R.B. Russel, *Amino acid properties and consequences of substitutions* in *Bioinformatics for Geneticsts*, M.R. Barnes, I.C. gray eds, Wiley, 2003. Although the book was published in 2003, much of the text of *Bioinformatics for Geneticists* contains information that has been published prior to the April 21, 1998 priority date of the present application, including the information provided at Exhibit B.

As indicated in Exhibit B, substitution preferences for tyrosine for all protein types including intracellular proteins into which CDKs would fall, are listed as Phe, Trp, or His. Thus, one skilled in the art would likely consider substituting Phe, Trp, or His for tyrosine in place of tyrosine, in the first instance. Leu, Cys, Val, Ile, and Met would also be considered since these amino acids are considered to constitute a neutral change for Tyrosine.

Again referring to Exhibit B, with respect to threonine, in all protein types, serine substitution is favored. However, since serine is also phosphorylatable (*see supra*), one skilled in the art practicing the present invention would not choose to substitute serine for a threonine. As indicated in Exhibit B, one skilled in the art wishing to substitute a non-phosphorylatable amino acid for threonine would in the first instance consider Ala, Asn, or Val. With respect to intracellular proteins, into which CDKs would fall, one skilled in

the art would also consider substituting for Threonine Cys, Asp, Glu, Lys, Met, His, Ile, Asn, Pro, Gln, Arg, Ser, Ala, or Val.

For example, Exhibit C provides an article by Hsieh J., et al. (1993) *J. Biol. Chem. 266*(20):15118-15126, involving studies using the human vitamin D receptor (hVDR) which is selectively phosphorylated by protein kinase C-β (PKD- β). Previous studies by Hsieh J. et al. (1991) had shown that the serine 51 residue of human VDR (hVDR), which is conserved in all known VDRs, is selectively phosphorylated by protein kinase C-β (PKD-β) *in vitro* and *in vivo.* The authors found that alteration of serine 51 to a non-phosphorylatable (e.g. glycine, aspartic acid, or alanine) residue resulted in an approximately 60% reduction in basal hVDR phosphorylation in intact cells. Mutation of serine 51 to threonine restored phosphorylation by PDK-B in vitro. Indeed, Hsieh J. et al. (1993) in selecting Gly, Asp, or Ala to substitute for Ser, were following known principles for amino acid substitution as e.g., complied in Exhibit B.

McGraw, T., et al. (1988) *Jour. Cell Biol (106)*:1061-1066, provided herewith at Exhibit D, also performed experiments where the major protein kinase C phosphorylation site, Ser-24 on human transferring receptor (TR) was replaced with a non-phosphorylatable amino acid, Gly. In this instance, the authors found that phosphorylation of Ser-24 is not required for receptor functioning. McGraw et al. therefore, were also following known principles for amino acid substitution as e.g., complied in Exhibit B.

Exhibit E provides a copy of an abstract by Orr, J.W., and Newton, A.C. (1994) *J. Biol. chem.. 269*(44): 27715-8. In this study, the authors produced mutants of protein kinase C where Thr500 was mutated to either an acidic residue (Glu) or a neutral, non-

phosphorylatable residue (Val). Substitution of Thr500 to Glu resulted in expression of a catalytically active protein kinase C in COS cells. In contrast, mutation of Thr500 to a neutral, non-phosphorylatable residue (Val) resulted in expression of an inactive enzyme.

Hung, D.J., et al. (April 15, 1998) *EMBO J. 17*(8):2308-18, provided herewith at Exhibit F also studied the *Drosophila* protein Fushi tarazu (Ftz), which is expressed sequentially in the embryo, beginning in alternate segments. During different developmental stages, the protein is heavily phosphorylated on different subsets of Ser and Thr residues. The authors demonstrated that in the embryo, mutagenesis of the Thr263 to a non-phosphorylatable residue Ala resulted in loss of ftz-dependent segments. Substitution of T263 with Asp which is also non-phosphorylatable, but which successfully mimics phosphorylated residues in a n umber of proteins, rescued the mutant phenotype.

The studies provided herewith at Exhibits C-F demonstrate that as of the priority date of this application, April 21, 1998, skilled artisans were well aware of different non-phosphorylatable amino acids that could be substituted for tyrosine, threonine, or serine.

Applicants further respectfully submit that although the present application exemplifies the present invention with respect to having both the tyrosine at position 15 and the threonine at position 14 in CDKA;1 substituted with non-phosphorylatable amino acid residues, the present application's broader teaching for use of a CDK comprising a non-phosphorylatable amino acid substituted for the relevant tyrosine has also been confirmed by the post-filing date literature. For example, Schuppler, U., et al. (1998) *Plant Physiol. 117*:667-678 (previously submitted as Schuppler Exhibit C with Applicants' response filed December 2, 2003), repeatedly refer to the role of tyrosine

phosphorylation in a plant CDK: *See eg.*, page 668, column 1, final sentence: "[w]e report evidence that water stress has rapid effects on Tyr phosphorylation and activity of a Cdc2-like enzyme and propose that these contribute to reduced cell-division activity"; page 675, column 2, final paragraph; "[i]nactivation of the kinase by stress within 3h was associated with an increase in the proportion of Tyr-phosphorylated protein, whereas the amount of protein remained constant"; page 677, final paragraph: "[w]e propose that water stress induces a signal that increases the phosphorylation of Tyr at the active site of Cdc2 kinase and that this results in a predominance of the inactive form of the enzyme, with consequence inhibition of progression into mitosis, which is dependent on high activity of the enzyme."

Moreover, as the remarks above and the Exhibits C-F show, the substitution of a non-phosphorylatable amino acid for a phosphorylatable amino acid at one position has been found to be sufficient in changing the activity of the relevant enzyme in many instances.

Finally, with respect to the written description rejection, the Examiner has taken the position that the specification allegedly does not provide sufficient guidance with respect to which PSTAIRE comprising cyclin-dependent kinases other than CDKA;1 could, when appropriately mutated, be used to confer drought or salt stress tolerance to a plant transformed therewith.

Applicants respectfully submit that the Examiner's position is not based on fact. The review article by Joubes, M., et al., (2000) *Plant Molecular Biology 43*:607-620 (submitted as Exhibit D with Applicants' response filed December 2, 2003), labels the plant gene family of CDKs having the PSTAIRE motif (as presently recited by the

claims) as the *CDKA* gene family. Page 68, final paragraph of Joubes et al., teach: "[t]he 31 plant CDKA have a highly conserved amino acid sequence, with 89% similarity (Figure 5). The fifteen most conserved residues of eukaryotic serine-threonine and tyrosine protein kinases are conserved in plant CDKA, including the residues involved in the ATP-binding site and in regulatory phosphorylation (Figure 5). The residues involved in defining the consensus phosphorylation site of CDK, i.e., SPXK, where S is the phosphoacceptor, are also conserved in plant CDKA (Figure 5)."

It is further submitted by Applicants that most, if not all of the genes encoding CDKs of the A type, set forth in Table 1of Joubes, M., et al., (2000), were available as of the priority date of the present application.

The foregoing remarks and exhibits demonstrate that the inventors had possession of the claimed invention as of the priority date of the present application. Withdrawal of the rejection of claims 1, 5-6, 10-11, 18-20, 22-26, 28-29 and 31 under the written description provision of 35 U.S.C. §112, first paragraph is therefore warranted.

Claims 1, 5-6, 10-11, 18-20, 22-26, 28-29 and 31 have also been rejected under 35 U.S.C. §112, first paragraph, as allegedly directed to non-enabled subject matter. According to the Examiner, the claimed invention is not fully enabled because the specification does not provide sufficient guidance with respect to which non-phosphorylatable amino acid residues other than alanine and phenylalanine may occupy the positions corresponding to residues 14 and 15 respectively in *Arabidopsis thaliana* CDKA;1.

In response to the position of the Examiner, Applicants repeat, reassert and incorporate by reference the argumentation provided above as well as Exhibits A-F,

which support Applicants' position that one skilled in the art having the present application as well as the literature extant as of the priority date of the application, would know which non-phosphorylatable amino acid residues to use in practicing the present invention.

The Examiner on page 5 of the Office Action also takes the position that the specification does not provide sufficient guidance with respect to how to use a PSTAIRE comprising cyclin-dependent kinase mutein having only the tyrosine corresponding to position 15 in *A. thaliana* CDKA;1 substituted with a non-phosphorylatable amino acid residue. Applicants repeat and reassert the argumentation set forth above, where it was submitted that the application is replete with such teaching, although plants comprising a CDK having both tyrosine and threonine substituted with non-phosphorylated amino acid residues are further *exemplified.* Applicants repeat and reassert that the law does not require a specific example of everything within the broad scope of the claims. *In re Anderson*, 471 F.2d 1237, 1240-41, 176 USPQ 331, 333 (CCPA 1973). Moreover, the law does not require *any* working examples. *In re Robbins*, 429 F.2d 452, 456, 166 USPQ 552, 555 (CCPA 1970).

In response to the Examiner's position that the specification does not provide sufficient guidance with respect to which PSTAIRE comprising cyclin-dependent kinases other than CDKA;1 could, when appropriately mutated, be used to confer drought or salt stress tolerance to a plant transformed therewith, Applicants repeat, reassert, and incorporate by reference the argumentation set forth above and supported by Joubes, M., et al., (2000) *Plant Molecular Biology 43*:607-620 (submitted as Exhibit D with Applicants' response filed December 2, 2003). Page 68, final paragraph of Joubes et al.,

teaches: "[t]he 31 plant CDKA have a highly conserved amino acid sequence, with 89% similarity (Figure 5). The fifteen most conserved residues of eukaryotic serine-threonine and tyrosine protein kinases are conserved in plant CDKA, including the residues involved in the ATP-binding site and in regulatory phosphorylation (Figure 5)." Most, if not all of the genes encoding CDKs of the A type, set forth in Table 1 of Joubes et al. (2000) were available as of the priority date of the present application.

According to the Examiner, the effect of changing the amino acid composition of a PSTAIRE comprising cyclin-dependent kinase on its ability to confer drought or salt stress tolerance when expressed in a plant is unpredictable. "Absent such guidance, it would require undue experimentation for one skilled in the art to determine which PSTAIRE comprising cyclin-dependent kinases to modify, and which non-phosphorylatable amino acid residues to use for their modification, in order to obtain nucleic acid molecules that would confer drought or salt stress tolerance to a plant transformed therewith. " Office Action, page 5, final sentence.

It is settled law that it is not necessary that every last detail of an invention be described, by working examples or otherwise. *Ex parte Wolter*, 214 USPQ 735 (Pat. Off. Bd. Appl 1979). In addition, "the patent need not teach, and *preferably omits*, what is well known in the art." *Hybritech, Inc. v. Monoclonal Antibodies, Inc.* 231 USPQ81 (Fed. Cir. 1986) (emphasis added).

It is respectfully submitted that as exhaustively discussed above, one skilled in the art would know which PSTAIRE comprising cyclin-dependent kinase other than CDKA;1 could be used (*see, e.g.*, Joubes et al.). Moreover, based on the teachings of the prior art a skilled artisan would know which non-phosphorylatable amino acids would be

suitable for substituting in a PSTAIRE comprising CDK. Exhibits C-F provide examples of prior art teachings where favored or neutral substitution preferences are taught.

Applicants further submit that even if the final choice of what non-phosphorylatable amino acid to substitute for the tyrosine or threonine in a plant CDK in order to render that plant drought or salt stress tolerant requires some experimentation, such experimentation is not fatal under the provisions of 35 U.S.C.§ 112, first paragraph.

A determination of what constitutes undue experimentation is not a simple factual determination, but rather is a conclusion reached by weighing many factual considerations. *In re Wands*, 858 F.2d 731, 737, 8 USPQ 1400, 1404 (Fed. Cir. 1988). Further, enablement is not precluded by the necessity for experimentation.

> The test [for undue experimtation] is not merely quantitative since a considerable amount of experimentation is permissible if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.

Id. (Emphasis added).

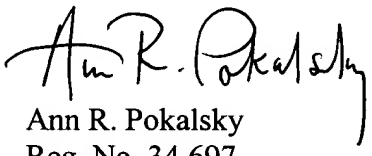
Accordingly, the foregoing remarks and the exhibits submitted herewith demonstrate that the determination of which PSTAIRE comprising CDK to modify would not entail undue experimentation. Further, the determination of which non-phosphorylatable amino acid residues to use for such modification in order to obtain nucleic acid molecules that would confer drought or salt stress tolerance would also not entail undue experimentation. Withdrawal of the rejection of claims 1, 5-6, 10-11, 18-20, 22-26, 28-29 and 31 under the enablement provision of 35 U.S.C. §112, first paragraph, is therefore warranted.

Claims 25-26, 28-29 and 31 have been rejected under 35 U.S.C.§102(b) as allegedly anticipated by Hemerly et al. (1995) *The EMBO J.14*(16):3925-3936. Hemerly et al. is cited for teaching a vector comprising a nucleic acid molecule encoding a cyclin-dependent kinase mutein comprising an alanine and a phenylalanine at positions corresponding to residues 14 and 15 respectively in *Arabidopsis thaliana* CDKA;1, wherein the nucleic acid molecule is operably linked to a constitutive CaMV 35S promoter, and transgenic *Arabidopsis* and tobacco plants comprising such nucleic acid molecule. The Examiner's position is that the constitutive CaMV 35S promoter used by Hemerly et al. is chimeric since it is spliced to a heterologous nucleic acid molecule.

As presently amended, claims 25 (and claims 26, and 28-29 dependent thereon) and claim 31 recite in relevant part a tissue-specific or abiotic stress-inducible promoter. As a chimeric promoter is no longer recited, withdrawal of the rejection of claims 25-26, 28-29 and 31 under 35 U.S.C. § 102(b) is respectfully requested.

In view of the forgoing amendments and remarks hereinabove, it is respectfully submitted that the present claims are in condition for allowance, which action is respectfully requested.

Respectfully submitted,



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